**Progetto di Ricerca:**

**Filling gaps in the biological control of *Fusarium* diseases of durum wheat and of related mycotoxins**

Wheat holds a significant economic relevance globally. Especially durum wheat (*Triticum durum* Desf.) represents one of the most relevant sources of food. In Italy, it contributed substantially with a production of 3.8 million tons in 2022. However, this crop is highly susceptible to the attack of fungal pathogens responsible for different severe diseases. Notably, the economic impact is significant, especially regarding the following major diseases: i) *Fusarium* Crown Rot (FCR) and *Fusarium* Foot Rot (FFR) are the most widespread diseases that appear already from the first stages of development of the plant, but the symptoms are observable throughout the entire vegetative cycle. The main pathogens belong to the *Fusarium* and *Microdochium* genera, and they can act individually or in combination, affecting primarily the roots, the crown, and the nodal/internodal portions of the stem. The main involved species are *F. culmorum, F. graminearum, M. nivale* var. *nivale* and *M. nivale* var. *majus; ii)* *Fusarium* head blight (FHB) is a global cereal disease caused by a complex of *Fusarium* species resulting in high yield losses and reduction in quality due to mycotoxin contamination of grain. The FHB community composition could be influenced by many factors, first and foremost climatic conditions, and for this reason, it is defined as a dynamic “complex” of species, in which each one *is* characterized by a specific mycotoxigenic profile. The main causal agents of this disease are considered the members of *Fusarium graminearum* species complex, which produces mainly trichothecenes, in particular Deoxynivalenol, Nivalenol, and their derivatives.

To date, the main defense strategies against these diseases are based on good agronomic practices and the use of fungicide-based chemical treatments. Within the PRIN PNRR 2022 Project (BICONTROVERSUM), a primary objective is to reduce the use of synthetic plant protection products for the previously mentioned wheat diseases. Instead, the project focuses on utilizing alternative solutions, primarily based on living biological entities known as Biological Control Agents (BCA). An innovative approach involves the use of antagonistic bacteria, acting both as a coating agent and a spray active ingredient in durum wheat protection. By significantly limiting the use of synthetic plant protection products like fungicides, it offers high sustainability. Importantly, it addresses a longstanding issue in the cereal sector: minimizing chemical residues and mycotoxin production.

**Piano di attività**

This research project stems from the necessity to integrate scientific research with a hands-on agronomic approach. The project's primary focus is to investigate the efficacy of antagonistic bacteria against various wheat pathogens, aiming to decrease the reliance on agrochemicals. This reduction not only mitigates the risk of food contamination but also contributes to environmental preservation. The primary goal is to develop a secure and dependable seed coating technique and bio-fungicide product for durum wheat utilizing beneficial bacteria.

The research will be conducted at the technical-scientific facilities of the Department of Agro-Food Sciences and Technologies (DISTAL), as well as at the Phytopathological Mycology Laboratories, which fall under the Plant Pathology Area. The research will also be carried out at the DISTAL greenhouses and the AUB facility, located in Cadriano.

In detail, the project will develop as follows:

* Investigating bacterial antagonistic activity against pathogenic fungi: the sequencing of the genomes of antagonistic bacterial strains will be performed and the subsequent bioinformatic analysis will help to identify potential antimicrobial peptides for inhibiting fungal pathogens; solid-state fermentation, that enhances peptide production using substrates derived from agro-industrial wastes, will be tested for the antimicrobial production enhancement in selected strains; antimicrobial peptides will be tested via agar diffusion assays against *Fusarium* and other indicator strains; additionally, a dual culture assay involving neutralized *Fusarium, Microdochium,* and other natural competitors will be conducted to enhance antimicrobial peptide production.
* *In vivo* tests on durum wheat using antagonistic bacteria against the main pathogens of *Fusarium* Rot (FCR/FFR): *in vivo* test will be conducted in controlled environment to assess the efficacy of individual antagonistic bacteria as seed coating agents after artificial inoculation of a conidial suspension of *F. culmorum/F. graminearum* and *Microdochium* spp. at soil level; three weeks after inoculation, symptoms at crown/root level will be evaluated using a disease symptoms scale: finally, identification of a control strategy based on a bacterial consortium in combating wheat FFT/FCR will be performed.
* *In vivo* test with antagonistic bacterial strains for their potential control of *Fusarium* Head Blght (FHB) on durum wheat: field trials will be conducted at the AUB facility (Cadriano) to evaluate bacterial strains' efficacy; selected strains will be tested in a two-year experiment in open-field conditions and are individually applied during 30% of flowering, including non-inoculated and inoculated controls, along with bacterial strains and reference fungicides; artificial inoculation with DON-producing *F. culmorum* and *F. graminearum* strains will be performed; visual observations, including incidence and disease severity, and DON quantification will be conducted to assess the impact of bacterial strains on FHB and DON management.
* Genome sequencing of the antagonistic bacterial strains will be conducted to identify potential pathogenicity and antimicrobial resistance genes, ensuring compliance with EFSA criteria; specific primers will be designed and validated for the two top-performing strains.
* Optimization of the growth medium for selected microorganisms using agro-food waste to enhance economic feasibility: waste and by-products from industries in the Emilia-Romagna region will be collected and characterized for total carbon, total nitrogen, and elemental analysis; culture media will be formulated based on chemical analyses, incorporating waste sources with optimal dilution and necessary micronutrients; laboratory-level growth will be compared with standard commercial media; selected strains' ability to maintain beneficial effects, such as antagonizing *Fusarium* and seed coating performance, in the formulated media will be assessed to ensure their effectiveness in soil.